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Wilcox, Ashleigh G; Buchan, Keith G.; Espino, Daniel

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*AG Wilcox[1], KG Buchan[2], DM Espino[1]**

[1]School of Mechanical Engineering, University of Birmingham, Birmingham, B15 2TT, UK

[2]Cardiothoracic Surgery, Aberdeen Royal Infirmary, Aberdeen, AB25 2ZD, UK

* Corresponding author:

Daniel M Espino

School of Mechanical Engineering

University of Birmingham

Birmingham, B15 2TT

UK

Tel: +44 (0)121 414 7355

fax. +44 (0) 121 414 3958

e-mail: d.m.espino@bham.ac.uk

ABSTRACT

This study aimed to characterise viscoelastic properties of different categories of chordae tendineae over a range of frequencies. Dynamic Mechanical Analysis (DMA) was performed using a materials testing machine. Chordae (n = 51) were dissected from seven porcine hearts and categorised as basal, marginal, strut or commissural. Chordae were loaded under a sinusoidally varying tensile load at a range of frequencies between 0.5-5 Hz, both at a standardised load (i.e. same mean load of 4 N for all chordae) and under chordal specific loading (i.e. based on *in vivo* loads for different chordae). Storage modulus and stiffness were frequency-dependent. Loss modulus and stiffness were frequency-independent. Storage and loss moduli, but not stiffness, decreased with chordal diameter. Therefore, strut chordae have the lowest moduli and marginal chordae the highest moduli. The hierarchy of dynamic storage and loss moduli is: marginal, commissural, basal and strut. In conclusion, viscoelastic properties of chordae are dependent on both frequency and chordal type. Future/novel replacement chordal materials should account for frequency and diameter dependent viscoelastic properties of chordae tendineae.

Key words: Chordae tendineae, dynamic mechanical analysis, mechanical properties, mitral valve, viscoelasticity.

1. Introduction

Viscoelastic properties of porcine mitral heart valve chordae tendineae have been measured *ex vivo* using Dynamic Mechanical Analysis (DMA). The storage and loss moduli, as well as stiffness, of different categories of mitral valve chordae have been determined. A frequency sweep was used at frequencies relevant to heart rates during rest, exercise and pathophysiology.

Chordae can be categorised as strut, basal, marginal or commissural (Al-Atabi *et al.*, 2012; Espino *et al.*, 2005; Lam *et al.*, 1970). Marginal chords insert into the edge of the leaflets, and basal chords insert away from the free edge of the leaflet between the free edge and the mitral valve annulus. Marginal chordae are thinner than basal chordae (Kunzelman & Cochran, 1990). Both the anterior and posterior leaflet of the mitral valve have marginal and basal chordae. Strut chordae are two thick basal chords that insert into the anterior leaflet. Chordae which branch so as to insert into both anterior and posterior leaflets at the two points on the annulus where they meet are termed commissural. Chordal categories are important as each have different roles in the mitral valve (Espino *et al.*, 2005; Obadia *et al.*, 1997). For example, posterior leaflet chordal failure has been quoted as being the main reason for mitral valve repair (Grande-Allen *et al.*, 2001) but anterior leaflet marginal chordal failure leads to more severe regurgitation (Espino *et al.*, 2005; Obadia *et al.*, 1997). Severe mitral regurgitation can be life-threatening and may require surgery (Carpentier, 1983; Galloway *et al.*, 2002; Maisano *et al.*, 1998).

Replacement of the valve with a prosthesis is one option, if surgery is necessary, another is surgical repair of the valve (Cohn *et al.*, 1988; Enriquez-Sarano *et al.*, 1995; Espino, 2007; Perier *et al.*, 1984). Chordal replacement with a synthetic chord has been particularly successful (Espino *et al.*, 2006a; Tomita *et al.*, 2002; Zussa *et al.*, 1997). However, there are concerns that the mismatch in stiffness could restrict valve motion

(Kobayashi *et al*., 1996). Tissue engineered chordae could be a future option for younger patients requiring surgery (Shi & Vesely, 2003 & 2004). A limitation for the development of novel biomaterials, be they synthetic or natural, is the limited knowledge of viscoelastic properties of different chordal categories.

Viscoelastic properties of a material are characterised by a storage and loss moduli (Aspden, 1991; Hukins *et al.*, 1999). The storage modulus characterises its ability to store energy which is then available for elastic recoil. The loss modulus characterises the ability of the material to dissipate energy. DMA is a method which can be used to characterise the frequency-dependent viscoelastic properties of biological tissues (Fulcher *et al*., 2009; Gadd & Shepherd, 2011) and synthetic materials (Mahomed *et al*., 2009; Wands *et al*., 2008).

Chordae are viscoelastic (Cochran & Kunzelman, 1991; Lim *et al*., 1977) and their mechanical properties depend on strain rate (Lim & Boughner, 1975) and diameter (Liao & Vesely, 2003). Different chordal categories have different material properties (Millard *et al*., 2011). While viscoelastic properties of chordae tendineae have been characterised (Lim *et al.*, 1977), variations amongst different chordal categories are not clear. Heart rate also varies with exercise and pathophysiology (Guyton & Hall, 1996). Therefore, frequency-dependent trends are important when determining chordal viscoelastic properties.

The aim of this project was to characterise the viscoelastic properties of chordae tendineae according to chordal category over a range of frequencies. The storage and loss stiffness/modulus have been measured over a range of loading frequencies. These properties have been determined at a standardised load to enable comparison between chordal categories, and compared to viscoelastic properties following loading specific to chordal categories.

2. Methods

2.1 Specimens

A total of 51 chordae were obtained from seven porcine hearts; a suitable model for the human heart (Kunzelman *et al*., 1994). Porcine hearts were obtained from a supplier (Fresh Tissue Supplies, East Sussex) who froze the hearts when excised and delivered them frozen and sealed. On arrival in the laboratory, hearts were individually wrapped in tissue paper, soaked in Ringer's solution and stored in a freezer at −40°C in heat-sealed bags. This followed protocols used in previous studies with mitral valves (Espino *et al*., 2005, 2006a, 2006b, 2007; Millard *et al*., 2011). When chordae were required for testing, samples were dissected from an intact heart, following a single thaw process. Chordae were identified as either: anterior leaflet marginal, basal or strut; posterior leaflet marginal or basal; or commissural. This categorisation is consistent with our previous studies (Espino *et al*., 2005; Millard *et al*., 2011).

2.2 DMA

A Bose 3200 materials testing machine (Bose Corporation, ElectroForce Systems Group, Minnesota, USA) operated by WinTest software was used for DMA. During tests the applied force and resulting displacement were measured. From these measurements the dynamic stiffness, k^*, (i.e. force to displacement ratio) was calculated along with the phase angle, δ, between the peak force and peak displacement (Haddad, 1995). The storage, k', and loss, k'', stiffness were then derived using equations 1 and 2, respectively (Pearson & Espino, 2013). k' and k'' are a measure of the ability of a viscoelastic structure to store and dissipate energy, respectively (Haddad, 1995).

$$k' = k^* \cos\delta \qquad 1$$

$$k'' = k^* \sin\delta \qquad 2$$

The storage, *E'* (equation 3), and loss, *E''* (equation 4), moduli are obtained by normalising *k'* and *k''*, respectively, for shape (Fulcher *et al*., 2009).

$$E' = \frac{k^* \cos\delta}{S} \qquad 3$$

$$E'' = \frac{k^* \sin\delta}{S} \qquad 4$$

Here, *S* is a shape factor dependent on the sample geometry tested. The shape factor for a cylinder of diameter *d* and length *l* is determined using equation 5 (Fulcher *et al*., 2009). We have previously found chordae to be approximately cylindrical (Millard *et al*., 2011).

$$S = \frac{\pi d^2}{4l} \qquad 5$$

The relationship between *E', E''* and a material's complex modulus, *E**, and δ is given in equations 6 and 7 (Hukins *et al*., 1999), respectively.

$$\left|E^*\right| = \sqrt{E'^2 + E''^2} \qquad 6$$

$$\delta = \tan^{-1}\left(\frac{E''}{E'}\right) \qquad 7$$

2.3 Frequency sweep for chordae

For testing, chordae were gripped using a similar method to that described previously (Millard *et al*., 2011). Briefly, chordae were held at either end by grips lined with fine sand-paper. When chordae were gripped, the test machine cross-head position was adjusted so that tests started when chordae were unloaded (controlled by monitoring the digital load display) but with excess slack removed. A standardised chord length of 5 mm was tested. Chordal diameter was measured, using digital calipers, in this position at four different locations along its length with an average taken (table 1). Measurements obtained along the chordal length tested were consistent with chordae being approximately cylindrical (Barber *et al*., 2001;

Liao & Vesely, 2003; Millard *et al*., 2011). During testing, chordae were maintained hydrated by having loosely wrapped tissue paper saturated in Ringer's solution around them.

A sinusoidally varying tensile force was applied to chordae at frequencies between 0.5-5 Hz, increasing in steps of 0.5 Hz. A rest period of 2 s, at the mean load, was included between testing at different frequencies. The range of frequencies used for testing covers:

- bradycardia down to 30 bpm (beats per minute), i.e. 0.5 Hz (Guyton & Hall, 1996);
- physiological heart rates of 60 – 70 bpm, i.e. 1.0 – 1.2 Hz (Guyton & Hall, 1996);
- exercise heart rates of up to 170 bpm, i.e. 2.8 Hz (Ghasemi-Bahraseman *et al*., 2013);
- tachycardia up to 300 bpm, i.e. 5 Hz (Guyton & Hall, 1996).

2.4 Loading protocols

Two loading protocols were used for each chord, with their order varied, termed *standardised* and *chordal specific*. Before each protocol, chordae were sinusoidally loaded for 200 cycles at 1 Hz under the tensile force for the respective protocol. This was used to ensure repeatability from one cycle to the next, and is equivalent to around 3 minutes worth of cycles in a beating heart.

Standardised loading. A sinusoidally varying tensile force, with a mean 4 N and dynamic amplitude of 2 N (i.e. between 3 and 5 N) was applied to all chordae. This protocol was used to standardise the load at which chordal viscoelastic properties were compared. Also, we have previously tested chordae at similar loads without inducing failure (Millard *et al*., 2011).

Chordal specific loading. Marginal chordae were sinusoidally loaded under a mean tensile force of 0.6 N with a dynamic amplitude of 0.5 N. Basal chordae (including strut chordae) were sinusoidally loaded under a mean tensile force of 0.8 N with a dynamic amplitude of 0.7 N. These loading parameters were estimated from studies which have measured the forces chordae are exposed to *in vivo* (Lomholt *et al.*, 2002; Nielsen *et al.*, 2005). Peak loading was linearly extrapolated to blood pressure of 150 mmHg as *in vivo* measurements were made in anesthetised animals with peak blood pressures of around 100 mmHg. Chordal tension has been found to increase linearly with ventricular pressure (Lomholt *et al.*, 2002). No values for the loading of commissural chordae were found, so the protocol for basal chordae was used.

2.5 Data analysis

Statistical comparisons of data obtained were made between chordal categories (section 2.1) and loading protocols (section 2.4). Complex moduli, phase angle, storage and loss moduli and stiffness were compared at all frequencies. A one-way analysis of variance (ANOVA) was undertaken using Tukey's method for multiple comparisons to investigate significant differences ($p < 0.05$) between chordal categories (Bland, 2000). For conciseness, comparisons at 1 Hz are presented, a reasonable physiological heart rate for a healthy individual (Guyton & Hall, 1996).

Significant differences ($p < 0.05$) were determined between matched data at different frequencies using paired t-tests. Significant differences ($p < 0.05$) in data obtained from standardised and chordal specific protocols were obtained using paired t-tests as each chord was tested under both protocols.

Significance of the correlation between storage, and loss, moduli to chordal diameter was assessed ($p < 0.05$). Standard non-linear regression equations (SigmaPlot v12.0, Dundas Software Ltd, Germany) were compared by assessing R^2 values. Regression equations provided are, therefore, the best fit for the empirical relationship between the viscoelastic property and chordal diameter.

3. Results

3.1 Standardised and chordal specific loading

There were significant differences in the complex moduli and phase angles of chordae at standardised and chordal specific loading protocols at 1 Hz. For all chordal categories, the complex modulus increased significantly under the higher standardised loading ($p < 0.05$). However, the complex modulus was chordal category dependent ($p < 0.05$; table 2). The mean complex moduli ranged from 0.33 (strut chordae) to 1.51 kN/mm^2 (anterior marginal chordae) for standardised loading. Under chordal specific loading the mean complex modulus ranged from 0.15 (strut chordae) to 0.86 kN/mm^2 (anterior marginal chordae).

For all chordal categories, the phase angle decreased significantly under the higher standardised loading ($p < 0.05$). However, no significant differences were determined between the phase angles of different chordal categories (table 2). The mean phase angle for chordae was 3.7° under standardised loading and 5.4° under chordal specific loading.

Storage and loss moduli and stiffness all increased significantly under the higher standardised loading ($p < 0.05$; table 3). However, overall trends with frequency were not affected. This is shown for a sample marginal (figure 1) and strut (figure 2) chord, where increased load for a single chord leads to an offset, but not change, in the trends for storage or loss modulus with frequency.

The complex modulus and phase angles correspond to storage and loss modulus and stiffness as described in section 2.2. Subsequent results (sections 3.2 – 3.4) focus on storage and loss moduli and stiffness.

3.2 Anterior versus posterior leaflet

No significant differences in mechanical properties were found between anterior and posterior leaflet chordae of the same categories (i.e. basal or marginal chordae) at 1 Hz under standardised loading ($p > 0.05$; table 2 & table 4). Therefore, similar values for storage and loss stiffness, and storage and loss modulus, were determined for the same category of anterior and posterior leaflet chordae (table 4).

Grouping anterior and posterior leaflet basal and marginal chordae led to significant differences in storage and loss moduli. Basal chordae had significantly lower storage and loss moduli when compared to marginal chordae ($p < 0.05$; table 5).

3.3 Storage modulus & stiffness

The storage modulus decreased significantly with chordal diameter ($p < 0.05$, $R^2 = 87\%$; figure 3). At 1 Hz, this non-linear decrease was described using a power equation (equation 8; figure 3). Anterior leaflet marginal chordae (1.51 kN/mm^2) had the highest and strut chordae (0.33 kN/mm^2) the lowest storage modulus at 1 Hz of loading (table 4). Empirically, experimentally obtained storage modulus, E' (N/mm^2), can be related to chordal diameter, d (mm), by

$$E' = 148.434d^{-1.7777}. \qquad 8$$

The storage modulus increased with frequency (figures 1 & 2). For example, for anterior leaflet marginal chordae it increased from 1.47 kN/mm^2 (at 0.49 Hz; table 6) to 1.63 kN/mm^2 (at 4.89 Hz; table 7). For strut chordae it increased from 0.32 kN/mm^2 (at 0.49 Hz; table 6) to 0.34 kN/mm^2 (at 4.89 Hz; table 7). Storage moduli at 4.89 Hz were significantly greater than those at 0.49 Hz ($p < 0.05$).

There were no significant differences in storage stiffness across chordal categories at 1 Hz (table 4). The mean storage stiffness was 19.2 N/mm for all chordae at 1 Hz. This increased significantly from 18.8 to 20.4 N/mm as frequency increased from 0.49 to 4.89 Hz ($p < 0.05$; table 8).

3.4 Loss modulus & stiffness

The loss modulus decreased significantly with chordal diameter ($p < 0.05$, R^2 = 74%; figure 4). At 1 Hz, this non-linear decrease was described using a power equation (equation 9; figure 4). Equation 9 is the empirical relationship between the experimentally obtained loss modulus, E'' (N/mm^2) and chordal diameter, d (mm).

$$E'' = 8.4368d^{-1.9094} \qquad 9$$

Marginal chordae (0.11 kN/mm^2) had the highest and strut chordae (0.02 kN/mm^2) the lowest loss modulus at 1 Hz (table 4). Loss modulus of chordae did not vary with frequency (figures 1 & 2; see also tables 6 & 7).

There were no significant differences in loss stiffness across chordal categories ($p > 0.05$; tables 4, 6 & 7). The mean loss stiffness was 1.2 N/mm for all chordae across all frequencies (tables 5 & 8).

4. Discussion

4.1 Overview

DMA has been used to characterise the viscoelastic properties of mitral valve chordae tendineae from porcine hearts. The dynamic stiffness, phase angle and shape factor were obtained for chordal categories, from which storage and loss stiffness and moduli have been derived. Storage and loss stiffness and moduli are used to explain our findings because they are a measure of the ability of chordae to store and dissipate energy, respectively. These parameters relate directly to spring and dashpot components of viscoelastic models (Haddad, 1995). The dependency of viscoelastic properties on chordal category, diameter and frequency have been investigated. It has been found that:

- there were no differences in viscoelasticity between comparable anterior and posterior leaflet chordae (i.e. basal or marginal chordae);
- increasing the applied loading did not alter frequency-dependent trends, however, it did increase the absolute value of parameters measured;
- storage modulus and stiffness were frequency-dependent, whereas loss modulus and stiffness were frequency-independent;
- storage and loss moduli, but not storage and loss stiffness, varied with chordal category which is consistent with previous measurements of Young's modulus and stiffness (Millard *et al*., 2011);
- storage and loss moduli both decreased with increased chordal diameter, therefore, strut chordae have the lowest moduli and marginal chordae the highest moduli.

4.2 Viscoelasticity

The viscoelasticity of chordae tendineae is established through measurement of their dynamics properties (Lim *et al*., 1977) and hysteresis (Cochran & Kunzelman, 1991; Ritchie *et al*., 2006). Lim *et al.* (1977) did not classify chordae according to chordal category. Cochran & Kunzelman (1991) measured hysteresis for basal and marginal chordae but did not provide quantitative measure of hysteresis. Ritchie *et al*. (2006) reported mid-load hysteresis to be 10% for strut chordae. However, neither study reported viscoelastic properties. Therefore, it is difficult to compare our chordal category viscoelastic properties.

Our results show that the storage modulus is greater than loss modulus over physiological frequencies. This is consistent with the findings from Lim *et al.* (1977). In their study, however, lower storage and loss moduli are reported than those reported in our study. They reported storage moduli in the range of 0.02 – 0.14 kN/mm^2, and loss moduli in the range of 0.01 – 0.07 kN/mm^2. Our values for loss modulus range from 0.02 – 0.11 kN/mm^2 for different chordal categories. However, our storage modulus values (at 1 Hz) ranged from 0.33 – 1.51 kN/mm^2. This may reflect the higher loading used in our study. We found that relatively small increases in loading led to large increases in storage modulus. For example, we found that increasing the mean load to 4 N doubled the storage modulus. Under chordal specific loads our range of storage modulus reduced to 0.14 – 0.82 kN/mm^2 (Wilcox & Espino, 2013). Further differences may be caused by differences in testing protocols; Lim *et al.* (1977) typically tested their specimens to a peak of 6.5% strain and tested human chordae.

In this study, a significant correlation was found between chordal diameter and storage and loss modulus. Such a correlation has been reported for the decrease in Young's modulus with increased chordal diameter (Liao & Vesely, 2003). It is also consistent with thinner chordae having higher storage and loss moduli (Lim *et al.*, 1977).

Our findings demonstrate that rate dependent changes in viscoelastic properties of chordae are caused by the frequency-dependent changes in storage modulus, but not loss modulus. Therefore, increased heart rate, e.g. during exercise (Ghasemi-Bahraseman *et al*., 2013), leads to more energy being stored in each chord that is subsequently used for recoil.

4.3 Ultrastructure and viscoelasticity

Collagen fibril diameter, interfibrillar linkages and crimp vary with chordal diameter (Liao & Vesely, 2003). The collagen fibril crimp period decreases from around 20 μm for thinner chordae to around 10 μm for thicker chordae, with a lower crimp period leading to greater extensibility. Thin chordae have smaller collagen fibril diameter and a greater number of interfibrillar linkages than thick chordae. These ultrastructural findings have been used to explain the greater tensile modulus of thin chordae (Liao & Vesely, 2003). This explanation would extend to the diameter dependent storage modulus of chordae, which also decreased with chordal diameter and varied with chordal category.

Glycosaminoglycans and proteoglycans have been found to be loading dependent across the mitral valve (Grande-Allen *et al.*, 2004) and may be important to collagen fibril interactions in chordae (Liao & Vesely, 2003). Proteoglycan infiltration may also reduce collagen fibril organisation in chordae (Whittaker *et al.*, 1987). Differences in proteoglycan levels could, thus, also contribute to thinner marginal chordae, exposed to high loading (Nielsen *et al.*, 1999), having a higher storage modulus than thicker basal and strut chordae.

Chordal categories consist of a similar percentage of collagen (Liao & Vesely, 2003) of around 65% dry weight content (Lis *et al.*, 1987). Collagen also aligns along the chordal length, with its crimp being straightened during loading (Millington-Sanders, 1998). These similarities could account for the loss modulus being independent of chordal category. However, the loss modulus is a measure the ability of a material to dissipate energy. Therefore, it is more likely dependent on fibre-matrix interactions and associated energy transfer and dissipation during loading (Aspden, 1994; Goh *et al.*, 2005).

Storage and loss stiffness were not found to be dependent on chordal category or diameter. These similarities may result from a balance between differences in diameter, glycosaminoglycans, proteoglycans, and/or collagen crimp (see above). The similarities in dynamic stiffness presumably aid smooth dynamic valve closure.

4.4 Chordae tendineae

Clinically, chordal diameter is not available to an operating surgeon. However, diameter can correspond to chordal categories (Liao & Vesely, 2003). Therefore, thinner marginal chordae having the highest and strut chordae the lowest storage and loss moduli. The hierarchy of chordal dynamic modulus is: marginal, commissural, basal and strut.

Differences in viscoelastic properties likely reflect chordal roles in the mitral valve: thinner marginal chordae being stiffer to ensure valve closure without regurgitation; thicker basal/strut chordae transfer greater loads but are not responsible for valve competence and can, thus, be more compliant under dynamic loading. The role of marginal chordae in preventing valve regurgitation and strut chordae in transferring higher proportion of loads is well established (Espino *et al*., 2005; Goetz *et al*., 2003; He *et al*., 2000; Lomholt *et al*.,2002; Messas *et al*., 2001; Nielsen *et al*., 1999 & 2003; Obadia *et al*., 1997; Timek *et al*., 2001).

Future studies into replacement should account for frequency of loading (i.e. heart rate) and category of chord being replaced. Novel replacement materials should replicate storage modulus dependency on diameter and frequency, and the loss modulus dependency on diameter.

Replacement studies would benefit from transient computational models that account for viscoelastic properties of chordae, including differences between chordal categories. Such models would enable assessment of replacement on maintaining physiological mitral valve peak strains of around 14% - 22% (Chen *et al*., 2004; Sacks *et al*., 2006) and peak stresses in the range of 0.25 – 0.60 MPa (Dal Pan *et al*., 2005; Einstein *et al*., 2005; Espino *et al*., 2013a & 2013b; Kunzelman *et al*., 1993; Lau *et al*., 2010; Votta *et al*., 2002). If one-dimensional spring elements are used to model chordae (Votta *et al*., 2002) then storage and loss stiffness should be applied (i.e. independent of chordal category).

4.5 Limitations

During frequency sweeps minor discrepancies occurred between the frequencies requested of the testing machine and the frequencies the machine applied to the sample. For example: when 0.5 Hz was requested the loading frequency applied was actually 0.49 Hz; whereas, when 5 Hz was requested, the actual frequency was 4.89 Hz (figures 1 and 2). The difference between actual experimental frequency and requested frequency did not exceed 2%. This minor discrepancy does not alter any of the conclusions from this study.

To maintain chordae hydrated during testing, they were loosely and lightly wrapped with small strips of light/thin/weak tissue paper which was saturated with Ringer's solution. The intention was that the tissue paper should act as a thin hydration layer to retain fluid when lightly sprayed with Ringer's solution during testing. The tissue paper was approximately an order of magnitude less in volume than the chordae. Therefore, we do not anticipate any large alternations to load/displacement measurements or subsequent conclusions. The method used for chordal hydration mimicked procedures used for connective tissues such as ligaments (Öhman *et al.*, 2009).

Alternative procedures to maintaining a thin hydration layer include testing chordae in a bathing solution or in air. However, performing DMA in a water bath is problematic because viscous damping is provided by the fluid in the bathing solution during actuator motion. This could lead to large errors in loss moduli and phase angle measurements. Whereas, during previous preliminary tests we have observed chordae to visibly stiffen when exposed to air over a few minutes. This would likely alter the storage moduli measured and phase angle.

5. Conclusion

Chordae tendineae are viscoelastic, with frequency and diameter dependent storage moduli and diameter dependent loss moduli. Chordal diameter is related to chordal category, leading to a hierarchy of chordal dynamic modulus of: marginal, commissural, basal and strut.

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TABLES

Table 1. Chordal diameter.

chordal category	*n*	diameter (mm)	
		mean	SD
Anterior marginal	8	0.31	0.12
Posterior marginal	9	0.33	0.06
Commissural	8	0.35	0.07
Anterior basal	8	0.41	0.11
Posterior basal	9	0.58	0.06
Strut	9	0.70	0.19

SD: Standard deviation;

n: number of samples tested from each region.

Table 2. Complex modulus and phase angle for chordal categories when loaded at 1 Hz under standardised and chordal specific loading.

Chordal category	*n*	Standardised Loading				Chordal Specific loading			
		Complex modulus (kN/mm^2)		Phase angle (°)		Complex modulus (kN/mm^2)		Phase angle (°)	
		mean	SD	mean	SD	mean	SD	mean	SD
Anterior marginal	8	1.51^{A}	0.92	4.5	2.7	0.86^{A}	0.60	5.5	2.2
Posterior marginal	9	$1.07^{A,B}$	0.48	3.7	1.6	$0.73^{A,B}$	0.44	5.9	2.4
Commissural	8	$0.91^{A,B,C}$	0.30	3.5	0.7	$0.49^{A,B,C}$	0.23	5.0	1.1
Anterior basal	8	$0.81^{A,B,C}$	0.45	3.3	0.5	$0.35^{B,C}$	0.21	5.2	1.1
Posterior basal	9	$0.52^{B,C}$	0.31	3.4	0.6	0.21^{C}	0.20	5.6	1.5
Strut	9	0.33^{C}	0.12	3.6	0.7	0.15^{C}	0.07	5.0	1.0

SD: Standard deviation;
The letters $^{A, B, C}$ are used to indicate significant differences between the different categories; where a category does not share a letter they are significantly different ($p < 0.05$);
n: number of samples tested from each region.

Table 3. Viscoelastic properties of all chordae when sinusoidally loaded at 1 Hz under standardised and chordal specific loading.

Loading criteria	n	Storage modulus (kN/mm^2)		Loss modulus (kN/mm^2)		Storage stiffness (N/mm)		Loss stiffness (N/mm)	
		mean	SD	mean	SD	mean	SD	mean	SD
Standardised	51	0.84*	0.60	0.06*	0.05	19.2*	4.6	1.21*	0.43
Chordal specific	51	0.46	0.41	0.04	0.05	9.8	5.9	0.95	0.65

SD: Standard deviation;
*denotes statistically significant difference ($p < 0.05$) between the two loading conditions;
n: number of samples tested from each region.

Table 4. Viscoelastic properties of chordal categories when sinusoidally loaded at 1 Hz under standardised loading.

chordal category	*n*	Storage modulus (kN/mm^2)		Loss modulus (kN/mm^2)		Storage stiffness (N/mm)		Loss stiffness (N/mm)	
		mean	SD	mean	SD	mean	SD	mean	SD
Anterior marginal	8	1.51^{A}	0.92	0.11^{A}	0.07	17.3	3.7	1.2	0.4
Posterior marginal	9	$1.06^{A,B}$	0.48	$0.07^{A,B}$	0.04	18.8	5.3	1.2	0.6
Commissural	8	$0.90^{A,B,C}$	0.29	0.06^{B}	0.03	18.7	3.4	1.2	0.4
Anterior basal	8	$0.81^{A,B,C}$	0.45	0.05^{B}	0.02	18.4	4.8	1.0	0.3
Posterior basal	9	$0.52^{B,C}$	0.31	0.03^{B}	0.02	21.2	4.6	1.3	0.4
Strut	9	0.33^{C}	0.12	0.02^{B}	0.01	20.5	5.7	1.3	0.6

SD: Standard deviation;
The letters $^{A,\ B,\ C}$ are used to indicate significant differences between the different categories; where a category does not share a letter they are significantly different ($p < 0.05$);
n: number of samples tested from each region.

Table 5. Viscoelastic properties of marginal and basal chordae when sinusoidally loaded at 1 Hz under standardised loading.

Chordal category		Storage modulus (kN/mm^2)		Loss modulus (kN/mm^2)	
	n	mean	SD	mean	SD
Marginal	17	1.27*	0.73	0.09*	0.06
Basal	17	0.66	0.40	0.04	0.02

SD: Standard deviation;
*denotes statistically significant difference between chordal categories ($p < 0.05$);
n: number of samples tested from each region.

Table 6. Viscoelastic properties of chordal categories when sinusoidally loaded at 0.49 Hz under standardised loading.

chordal category	*n*	Storage modulus (kN/mm^2)		Loss modulus (kN/mm^2)		Storage stiffness (N/mm)		Loss stiffness (N/mm)	
		mean	SD	mean	SD	mean	SD	mean	SD
Anterior marginal	8	1.47^{A}	0.89	0.12^{A}	0.08	16.8	3.7	1.3	0.4
Posterior marginal	9	$1.04^{A,B}$	0.47	$0.07^{A,B}$	0.04	18.3	5.0	1.2	0.5
Commissural	8	$0.89^{A,B,C}$	0.30	0.05^{B}	0.03	18.3	3.3	1.1	0.4
Anterior basal	8	$0.80^{A,B,C}$	0.45	0.05^{B}	0.02	18.2	5.1	1.1	0.3
Posterior basal	9	$0.51^{B,C}$	0.30	0.03^{B}	0.02	20.6	4.5	1.3	0.4
Strut	9	0.32^{C}	0.11	0.02^{B}	0.01	20.0	5.4	1.3	0.7

SD: Standard deviation;
The letters $^{A, B, C}$ are used to indicate significant differences between the different categories; where a category does not share a letter they are significantly different ($p < 0.05$);
n: number of samples tested from each region.

Table 7. Viscoelastic properties of chordal categories when sinusoidally loaded at 4.89 Hz under standardised loading.

chordal category	*n*	Storage modulus (kN/mm^2)		Loss modulus (kN/mm^2)		Storage stiffness (N/mm)		Loss stiffness (N/mm)	
		mean	SD	mean	SD	mean	SD	mean	SD
Anterior marginal	8	1.63^{A}	0.99	0.12^{A}	0.08	18.5	3.5	1.3	0.5
Posterior marginal	9	$1.12^{A,B}$	0.54	$0.07^{A,B}$	0.05	19.8	6.2	1.2	0.6
Commissural	8	$0.96^{A,B,C}$	0.30	0.06^{B}	0.03	20.1	4.2	1.2	0.4
Anterior basal	8	$0.86^{A,B,C}$	0.48	0.05^{B}	0.03	19.5	5.5	1.1	0.3
Posterior basal	9	$0.55^{B,C}$	0.32	0.03^{B}	0.02	22.4	4.9	1.3	0.5
Strut	9	0.34^{C}	0.12	0.02^{B}	0.01	21.6	6.5	1.2	0.5

SD: Standard deviation;
The letters $^{A, B, C}$ are used to indicate significant differences between the different categories; where a category does not share a letter they are significantly different ($p < 0.05$);
n: number of samples tested from each region.

Table 8. Comparison of dynamic stiffness at 0.49 Hz and 4.89 Hz.

		0.49 Hz		4.89 Hz	
	n	mean	SD	mean	SD
Storage stiffness (N/mm)	51	18.8	4.5	20.4*	5.2
Loss stiffness (N/mm)	51	1.2	0.5	1.2	0.5

SD: Standard deviation;
*denotes statistically significant difference ($p < 0.05$) between the two frequencies;
n: number of samples tested from each region.

FIGURE CAPTIONS

Figure 1. Frequency-dependency of storage (circles) and loss (squares) modulus for an anterior leaflet marginal chord. The chord was tested under standardised (black markers) and chordal specific (grey markers) loading.

Figure 2. Frequency-dependency of storage (circles) and loss (squares) modulus for an anterior leaflet strut chord. The chord was tested under standardised (black markers) and chordal specific (grey markers) loading.

Figure 3. Chordal diameter dependency of storage modulus at 1 Hz, under standardised loading. A power equation (equation 8) was fitted to the experimental data (R^2 = 87%; $p < 0.05$) as shown (solid line). The 95% confidence intervals are also included (dashed line).

Figure 4. Chordal diameter dependency of loss modulus at 1 Hz, under standardised loading. A power equation (equation 9) was fitted to the experimental data (R^2 = 74%; $p < 0.05$) as shown (solid line). The 95% confidence intervals are also included (dashed line).